International Biodeterioration & Biodegradation 59 (2007) 69–71

INTERNATIONAL BIODETERIORATION & BIODEGRADATION

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Short communication

Effect of natural products on gut microbes in Formosan subterranean termite, *Coptotermes formosanus*

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Received 27 February 2006; received in revised form 30 May 2006; accepted 10 June 2006
Available online 24 August 2006

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Abstract

The ability of three natural products (neem extract, capsaicin, and gleditschia) to reduce the number of Formosan subterranean termite (FST) hindgut microbes was investigated. FSTs were placed in Petri dishes containing a food source soaked in one of these extracts/compounds. The numbers of three flagellated protozoan species that inhabit the FST hindgut (*Pseudotrichonympha grassii*, *Spirotrichonympha leidyi* and *Holomastigotoides hartmanni*) and spirochaetes were counted over a defined period and analysed for changes in abundance. The results indicated that the neem extract was capable of reducing the population of *P. grassii* and spirochaetes. Exposure to this extract resulted in significant FST mortality. However, gleditschia extract and capsaicin did not reduce the FST gut microbial population at the concentrations used in this study.
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Keywords: Formosan subterranean termite (FST); Spirochaetes; Flagellates; Protozoans; Neem extract; Capsaicin

The Formosan subterranean termite (FST), *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), was first detected in the US in 1965 in Houston, TX (Woodson et al., 2001). Since then, the FST has spread rapidly throughout the southeastern US mainly by anthropogenic means such as transport of infested railroad ties, pallets, utility poles and boats (Woodson et al., 2001). The total economic loss due to FST in the US has been estimated at 1.7 billion dollars per year (Gold et al., 1996).

FSTs receive the bulk of their nutritional requirements from cellulosic materials, mainly wood in varying stages of decomposition, and have endogenous cellulases located in the anterior portion of the gut. Yet these cellulases can only partially break down cellulose. Researchers theorize that the cellulose not hydrolyzed in the anterior portion of the gut by the endogenous cellulases can be endocytosed and fermented by the symbiotic protists in the hindgut of lower termites (Inoue et al., 1997; Itakura et al., 1997; Nakashima et al., 2002). To fully digest cellulose, termites require the aid of cellulolytic flagellate protists found in the hindgut. The relationship between the termites and these protists is obligate and is nutritional symbiosis (Cook and Gold, 1998). The symbiotic flagellated protists found in the termite gut belong to the Phylum Parabasalia, one of the most primitive groups of eukaryotes. Parabasalids function in anaerobic environments and lack mitochondria. Instead of mitochondria, parabasalids have hydrogenosomes, which are anaerobic energy-generating organelles producing acetate, H_2, and CO_2. Three species of flagellated protists are found in the hindgut of FST, viz. *Spirotrichonympha leidyi*, *Holomastigotoides hartmanni*, and *Pseudotrichonympha grassii* (Ohkuma et al., 2000). Anaerobic bacteria harboured in the termite hindgut include acetogenic and nitrogen-fixing spirochaetes, and methanogens. The current understanding of cellulose digestion suggests that in breaking down cellulose the protozoans produce acetate, H_2 and CO_2. The acetogenic spirochaetes convert the H_2 and CO_2 into acetate. The acetate formed by acetogenic spirochaetes is a major carbon and energy source for termites (Leadbetter et al., 1999). Termites have a nitrogen-poor diet and must rely on nitrogen-fixing

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0964-8305/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibiod.2006.06.023

microbes to supplement their nitrogen input. Recent studies suggest that symbiotic nitrogen fixation may supply up to 60% of the nitrogen in termite biomass (Lilburn et al., 2001). Methanogens are believed to enhance cellulose digestion by their H_2 consumption (Shinzato et al., 1999).

Among the current termite control methods, one approach has been to limit growth using chitin synthesis inhibitors such as hexaflumron and azadirachtin (Hikawa et al., 2000; Rojas and Morales, 2001; Stansly et al., 2001; Salehzadeh et al., 2003). Limiting termite growth through the destruction of the gut microbes is a control method that has shown some success (Adams, 2004). The objective of this study was to determine the effects of three natural products at low concentration on the gut microbes of FST. The substances used were neem extract, capsaicin and gleditschia. The active ingredient in neem extract, azadirachtin, is a chitin synthesis inhibitor (Bilker et al., 2002; Salehzadeh et al., 2003). Capsaicin is an alkamide extracted from cayenne pepper, *Capsicum* spp. (Cichewicz and Thorne, 1996; Jones et al., 1997; Molina-Torres et al., 1999; Kurita et al., 2002). Gleditschia is an extract from the seed of *Gleditschia triacanthos*, a small, thorny tree found in the eastern and central US. The extract contains two alkaloids, gleditschine and stenocarpine. Gleditschine has been found to cause stupor and loss of reflexes and stenocarpine has been used as a local anaesthetic (Adams, 2004).

For the experiments, FST workers were collected in bucket traps placed in four different locations in New Orleans. The natural products, neem extract, capsaicin, and gleditschia were provided by the US Department of Agriculture, FST Research Unit, Southern Regional Research Center, New Orleans, LA, USA.

The experiments were set up in 50-mm Petri dishes (9 mm deep) with a 100-mg piece of filter paper to which the natural products had been applied. For application, the neem extract dissolved in methanol was used at a concentration of 0.15 ppm; capsaicin also dissolved in methanol was used at 0.132 ppm; and gleditschia in deionized water (DI) at 0.15 ppm. Methanol, ethanol and DI water were applied in controls. Each dish contained 20 workers and two soldiers, mimicking the natural composition of termite populations. Prior to the experiment, random samplings of gut microbial populations in these termites were found to be similar.

At 2-day intervals from zero time to day 10, the microbes in the gut of two FSTs from each Petri dish were counted using a bright-line haemacytometer (Sigma, St. Louis, MO, USA). The gut of each FST was opened in 100 μL saline solution using fine tipped forceps and the contents then gently mixed with the saline. Each side of the haemocytometer received 10 μL mixture, and *P. grassii*, *S. leidyi* and *H. hartmanni* were counted at 100 × magnification, and unspeciated spirochaetes at 400 ×, using a Nikon Model E-200 bright field microscope (Nikon Instruments Co., Houston, TX, USA). The data were analysed by comparing the initial and final mean population counts in a two-tailed paired *t*-test (Zar, 1999).

Since neem extract was found to reduce numbers of some microbial populations, a further experiment was conducted as above with neem concentrations up to 1 ppm. Termite mortality was determined from counts of workers over 3 weeks, but microbial counts were not made.

The experiments to control the protozoans and spirochaetes produced mixed results. Capsaicin had no effect on the counts (data not shown). Neem extract significantly lowered the number of *P. grassii* ($p = 0.01$; Fig. 1a) and spirochaetes ($p = 0.02$; Fig. 1b), but had no effect on *S. leidyi* and *H. hartmanni*. Gleditschia did not alter the abundance of any of the gut microbes.

For insects, the active ingredient in neem extract, azadirachtin, is a hormone disruptor (Schaaf et al., 2000; Bilker et al., 2002; Salehzadeh et al., 2003). The alkamide capsaicin has shown neurotoxic and antimicrobial properties in mammals (Cichewicz and Thorne, 1996; Jones et al., 1997; Molina-Torres et al., 1999; Kurita et al., 2002). Gleditschia contains stenocarpine and gleditschin, which have been found to cause stupor and loss of reflex activity in frogs (Adams, 2004).

The cause of the decrease in *P. grassii* and spirochaete numbers with neem extract may well have been the

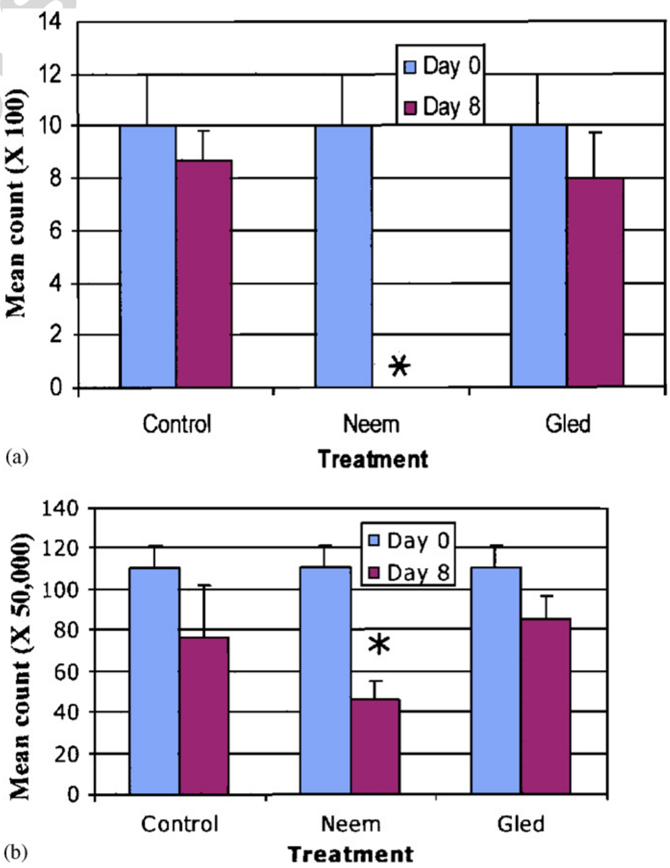

Fig. 1. Effect of neem extract and gleditschia on: (a) *Pseudotrichonympha grassii* and (b) spirochaete numbers in the hindgut of Formosan termites from four locations. Asterisk denotes significant difference from control ($p < 0.05$).

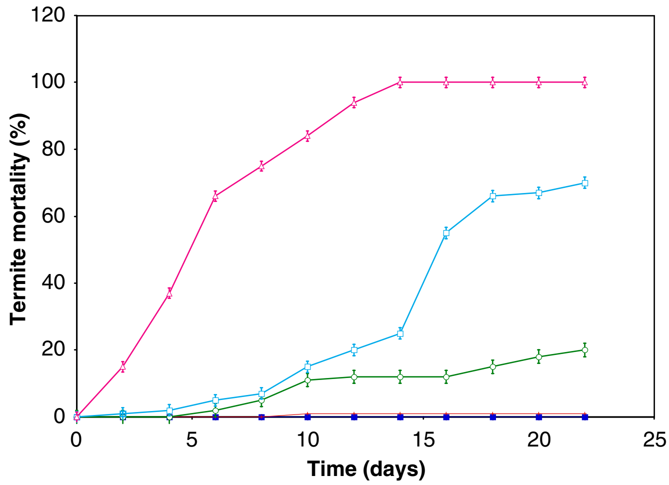

Fig. 2. Effect of neem extract on Formosan subterranean termite mortality with time: (+) 0.30 ppm; (○) 0.50 ppm; (□) 0.75 ppm; (△) 1 ppm neem extract (no mortality observed for control or 0.15 ppm neem extract).

ingredient azadirachtin. This would conflict with the report of Adams (2004), who used 99% pure azadirachtin (Sigma). A possible explanation of the disparity could be that some unknown compound(s) in the neem extract synergized with the azadirachtin, enhancing its effect.

In the experiment with different concentrations of neem extract, there was FST mortality at higher concentrations of the extract (Fig. 2). There were no deaths in controls or neem extract at the 0.15 ppm level, but there was mortality with $\geqslant$0.50 ppm. At 1 ppm there was 100% mortality within 14 days, and at 0.50 and 0.75 ppm neem extract, mortalities were significantly different from the controls. This preliminary study showed that potentially the neem extract could be used to control FST. The challenge is to design the formulations for delivery of neem extract as a treatment method.

Acknowledgment

This work was funded by a Specific Co-operative Agreement (58-6435-2-0103) with the USDA-ARS-SRRC, New Orleans, USA.

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